January 7, 2003

Jane Smith
Designated Federal Official
Endocrine Disruption Methods Validation Subcommittee
Office of Science Coordination and Policy/OPPTS
Room 4106-M
Mail Code 7201M
U.S. Environmental Protection Agency
Ariel Rios Building
1200 Pennsylvania Avenue NW
Washington, DC 20460

Dear Ms. Smith:

The following are comments that evolved from the December 4, 2002, EDMVS phone conference. The comments are in response to four questions submitted to the Committee by the Agency and in response to segments of the discussion that took place during the meeting.

1. Does EDMVS agree that the two-generation method recommended and applicable to four species of fish is appropriate? NO.

Several EDMVS panel members suggested that the EPA should conduct a side-by-side comparison between the two-generation study and the full and partial life cycle protocols. This is a critical exercise, but the side-by-side comparison also needs to include the multigenerational study. Only by conducting the complete set of side-by-side comparisons will the EPA be in a position to determine whether *in ovo* exposure of parental generation animals provides additional information (such as low dose sensitivity) compared to other proposed protocols.

In order to prevent having to go back and redo the 2 generation study later in order to replicate real-life exposure and to be able to look at the integrity of the F2 generation, it is imperative that the P generation is exposed from the egg stage through to the adult and allowed to reproduce, the F1 allowed to mature and reproduce, and the integrity of the F2 animals observed. EPA should take advantage of the additional information that can be gained by extending the exposure in the preliminary design. By doing this, EPA could avoid exposing more animals in the future to redo the 2-generation assay as written because exposure did not replicate real-world exposure. This will eliminate the need for repeated assays, each one a little more embellished than the previous.

2. Does the EDMVS agree that prevalidation should evaluate the increased sensitivity of a two-generation design over the existing full fish-life cycle standard practice? YES.

Again, as noted above, the EPA should also evaluate potential increased sensitivity of the multi-generational study, in addition to the two-generation, compared to the full fish-life cycle. The question of potential increased sensitivity added by new protocols will never be addressed unless the appropriate comparisons are conducted.

World Wildlife Fund
1250 Twenty-Fourth St., NW Washington, DC 20037-1132 USA
Tel: 202.293.4800 Fax: 202.887.5293
www.worldwildlife.org
Affiliated with World Wide Fund for Nature

However, EPA would make a serious mistake if it used this assay to define only population effects. In this trial effort, EPA must be alert not to miss the most sensitive endpoint--and endpoints that might be expressed early in the study in the P or F1 generation which could eventually reduce the number of animals needed and shorten assay time. It should be the goal of this Tier II assay to use the most sensitive species and ultimately reduce the scope of the assay and the number of animals required.

3. Does the EDMVS agree that prevalidation should demonstrate the sensitivity and reproducibility for each species in the recommended protocol? YES.
Unfortunately, during the meeting the discussion kept side-stepping the importance of using this prevalidation study to learn as much as possible about mechanisms of action. EPA should keep in mind the need to conserve the number of animals to be exposed and take advantage of every animal at this stage of development of the assay in order to avoid having to replicate any part of this assay again. Admittedly, there are many data gaps concerning hormones in fish. EPA would be remiss if it did not use every opportunity it can with these animals to fill in these data gaps.

It is also important to do the histology of the thyroid as well as the histology of the gonads. This was not proposed in the Draft Proposal.

WWF is concerned about using lethality as the endpoint in the range finding test. An endpoint as crude as this would miss the more subtle effects that EDSP should be addressing.

4. Does the EDMVS have suggestions to improve the DRP? YES. What follows should be considered in the preparation of future DRPs.

The Fish Life Cycle DRP (Tier II) is an excellent treatise on what is known about the developmental biology of fish. However, for the purpose of the EDMVS, it left too many unanswered questions. That is, it could have provided even more information than it did, rather than leaving so many questions unanswered. Throughout the meeting the questions posed by members of the committee should provide guidance for EPA for the preparation of future DRPs. The EDMVS members should have been informed up front that the Fish Life Cycle assay was being considered as a replacement for the fish pesticide assay. And in turn, several pages devoted to comparing the two assays would have been helpful.

In order to improve the utility of the DRP, it would be useful to have various paragraphs or sections end with comments on the policy and regulatory implications of what was presented.

In another instance, the rationale for providing a comprehensive literature search on 4 fish species should have been up front, or at least somewhere in the document. The most compelling arguments for using 4 species in the prevalidation stage were not in the DRP.

The following comments arose from the conversation that took place during the meeting. Some are in response to recommendations that were presented from the members:

It was suggested that an extensive search for missing data on fish exposure studies should be undertaken using proprietary data (registrants' data.)

It probably would not be an effective use of time to try to compare the historical literature results from a retrospective database. These data likely would not be helpful for EDMVS because the endocrine endpoints studied in the past would in all probability be apical, not the sensitive end points of concern to EDSP. However, should EPA decide to engage in such a search, the proprietary literature to be inventoried should be open to the public. In addition, the academic literature should be considered, because it is here where the critical endpoints of concern are being studied extensively. Most of what is known about endocrine effects in wildlife has come from academicians. This resource should be given equal weight in all considerations.

If any literature searches are going to be done, the lowest NOECs should be compared among the historical and current studies regardless of sector (academic, government, industry).

It was emphasized during the meeting that EPA would be making a serious mistake if a full two generation assay is not done.

The assay as now presented truncates exposure, exposing the P generation only when they reach adulthood rather than from the egg stage. After the disadvantage of the 2-generation incomplete exposure scenario was addressed several times during the meeting, the facilitator said that we (meaning EPA) would move forward with the 2-generation assay as presented as if it was a foregone conclusion. I would argue that there was enough concern about the truncated exposure of the adults in the 2-generation assay for EPA to reconsider whether, in the end, the 2-generation study as presented now would be a wise choice. The assay must start with the P generation exposed as eggs and follow their offspring through to maturity in the F2 generation as described in the multigenerational protocol.

Questions not posed during the meeting

At an earlier EDMVS meeting we discussed the possibility of using a fish assay as a Tier I *in vivo* estrogen/androgen/thyroid (EAT) assay and eliminating other screens. Has any consideration been given to using this assay for that purpose as well?

Sincerely yours,

Theo Colborn, Ph.D
Director
Wildlife and Contaminants Program